crickets has yet to be examined. Amano (2017) demonstrated no difference in AN-2 response at 3-days post deafferntaion but could not examine the difference between males and females at 18-20 days post deafferentaion due to an absence of response of AN-2 to all sound stimuli in females. Amano (2017) noted swollen abdomens, indicative of egg storage, in the 18-20 post deaffferentation females and suggested a potential role of hormones in female unresponsiveness. Hormonal changes within the female occur as she undergoes a life history trade off and begins to focus energy on reproduction over mate finding or predator avoidance (Zera and Cisper, 2001; Zera, Sall, and Grudzinski, 1997). For this reason, less time is spent in the air flying around leaving her exposed to predators and thus the importance of auditory input decreases.

The objectives of this project are to use an electrophysiological approach to further characterize the functional implications of the morphological differences seen in previous research. After learning the dissection, we examined the function of morphological changes by recording from the AN-2 nerves of male and female crickets 15- days post-deafferentation who were raised in isolation from the opposite sex. The aim of this project was to examine the role of hormones in female deafness, explored by raising crickets in isolation from the opposite sex and testing the responsivity of AN-2 at an earlier time point post deafferentaion. The number of action potentials produced in response to the stimulus and the timing of the action potentials were used to quantify the recovery and functionality of AN-2.

Female and male Common Mediterranean field crickets, , were acquired from an inbred colony at the Hoy Lab (Cornell U, Ithaca, NY) and housed in rooms at 70-80% humidity and 20-25 C on a 12L:12D cycle and fed commercial cat chow and water . Housing conditions varied slightly from previous work (Amano, 2017) where crickets were housed at 40-60% humidity and 28 C. Additionally, daylight conditions were reversed so that it was dark for the crickets during the day to change the circadian rhythm of the crickets to increase responsivity of AN-2 Crickets were isolated as 7th and 8th instars until they molted into adults. They were further isolated for 17 days. Two days after their initial molt, crickets were temporarily removed from their cage for a deafferentation procedure where the right foreleg was cut at the tibiofemoral joint. A control procedure was performed, leaving the auditory system intact but subjecting the cricket to the stress of the procedure by severing the leg between the tarsus and claw.

15-day post-deafferentation male and female, control and deafferented crickets were cooled for 20 minutes on ice to immobilize them. Following the removal of the cricket's wings, mesothoracic and metathoracic legs, crickets were positioned, using hot wax (50% cello rosin, 50% beeswax), on to a magnetic block ventral side up. Further immobilization was accomplished using wax to ground the coxa to the back of the head and the claw of the intact foreleg to the coxa, insuring the exposure of the tympannal membrane. Crickets were positioned to mimic their body position in flight because of the context dependent response of the auditory system to sound stimulus (Nolen and Hoy, 1984; Hofstede et al., 2009). Wax was then placed over the mouth of the cricket until air consumption was inhibited. After immobilization, the prothoracic plate, esophagus, trachea and gut were removed to expose the neck connectives. The neck cavity was filled with saline (140 mM NaCl, 5mM KCl, 7 mM CaCl2, 1 mM MgCl2, 5 mM TES, 4 mM NaHCO3, 5mM Trehalose, pH 7.3) and each connective was desheathed by peeling back the sheath from the lateral side of each connective to minimize damage to AN-2 located on the ventral medial quadrant of the neck connective.

Physiological recording and sound stimuli

An electrophysiological set up was used to record extracellularly from exposed neck connectives. Silver hook electrodes were placed so that AN-2 rested on each electrode, saline was removed and electrodes were isolated with petroleum jelly. To decrease ascending and descending spontaneous

activity, unrelated to AN-2 activity, neck connectives were cut at the head and between the mesosternite and metesternite chest. All recordings were performed in an egg carton mattress foam lined box to prevent interference of extraneous noise. AN-2 responsivity was measured in response to stimulated sound sweeps, created with SciLab4.1 scripts (Digiteo; Le Chesnay Cedex, France) and consisting of 3, 5, 7, 8, 10, 12, 15, 18, 20, and 22kHz frequencies. For every frequency, intensities of 5 db increments ranging from 40-95db were played in triplicate. Each pulse was 35ms with 1 sec interpulse period in between and increased and fell for 5ms. The sweep was played using speakers (Motorola/CTS piezoelectric tweeters, KSN1165A; frequency response 2-30 kHz). All responses were amplified using an A-M Systems differential AC amplifier Model 1700 (A-M Systems, Inc.; Carlsborg, WA) and recorded with a PC computer using a CED Micro 1401 board and the computer software Spike2, version 7.17 (Cambridge Electronic Design; Cambridge, UK).